5





CLAIMS

What is claimed is:

A method for protecting an animal subject against lethal infection with *B. anthracis*, comprising:

administering an immunogenic composition which comprises purified or recombinant B. anthracis lethal factor (LF) protein or an immunogenic fragment thereof to the subject.

2. The method of claim 1 wherein the immunogenic composition comprises a mutated LF protein or an immunogenic fragment of an LF protein, and

further comprising administering an immunogenic composition which comprises purified or recombinant *B. anthracis* protective antigen(PA) protein or an immunogenic fragment thereof to the subject.

- 3. The method of claim 1 wherein the LR protein comprises a sequence which is at least 90% identical to a sequence extending from amino acid 1 through amino acid 775 of the sequence set forth in SEQ ID NO:2.
- 4. The method of claim 1 wherein the LF protein fragment comprises a sequence which is at least 90% identical to a sequence extending from amino acid 9 through amino acid 252 of the sequence set forth in SEQ ID NO. 2.
- 5. The method of claim 2 wherein the PA protein comprises a sequence which is at least 90% identical to a sequence extending from amino acid 1 through amino acid 735 of the sequence set forth in SEQ ID NO. 4.
- 6. The method of claim 2 wherein the PA protein fragment comprises a sequence which is at least 90% identical to a sequence extending from amino acid 175 through amino acid 735 of the sequence set forth in SEQ ID NO. 4.
- 7. A method for protecting a susceptible animal subject against lethal infection with *B. anthracis*, comprising:

25

administering a nucleic acid-based immunogenic composition which comprises an isolated polynucleotide which encodes a mutated *B. anthracis* lethal factor (LF) protein or an immunogenic fragment thereof to the subject, said polynucleotide being operably linked to a promoter which drives expression of the mutated LF protein or the immunogenic LF protein fragment.

- 8. The method of claim 7 further comprising administering an immunogenic composition which comprises an isolated polynucleotide which encodes *B. anthracis* protective antigen(PA) protein or an immunogenic fragment thereof to the subject, said polynucleotide being operably linked to a promoter which drives expression of the PA protein or immunogenic fragment thereof in the subject.
- 9. The method of claim 7 wherein the LF protein comprises a sequence which is at least 90% identical to a sequence extending from amino acid 1 through amino acid 775 of the sequence set forth in SEQ ID NO:2.
- 10. The method of claim 7 wherein the LF protein fragment comprises a sequence which is at least 90% identical to a sequence extending from amino acid 9 through amino acid 252 of the sequence set froth in SEQ ID NO. 2.
- 11. The method of claim 8 wherein the PA protein comprises a sequence which is at least 90% identical to a sequence extending from amino acid 1 through amino acid 735 of the sequence set forth in SEQ ID NO. 4.
- 12. The method of claim 8 wherein the PA protein fragment comprises a sequence which is at least 90% identical to a sequence extending from amino acid 175 through amino acid 735 of the sequence set forth in SEQ ID NO. 4.
- 13. The method of claim 7 wherein the polynucleotide is a component of a nucleic acid-based vaccine
- The method of claim 7 wherein the polynucleotide is a component of a viral vaccine.

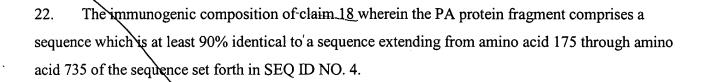
5

- 15. The method of claim 8 wherein administration of the LF polynucleotide and the PA polynucleotide enhance production of antibodies to LF and PA protein in the subject.
- 16. The method of claim 8 further comprising administering a peptide-based immunogenic composition to the subject, said second immunogenic composition comprising an immunogen selected from the group consisting of a mutated LF protein, an immunogenic fragment of an LF protein, a PA protein, an immunogenic fragment of a PA protein, and combinations thereof, wherein said second immunogenic composition is administered to the subject before or after administration of the polynucleotide-based immunogenic composition

An immunogenic composition for preparing a vaccine which protects a subject against lethal infection *B. anthracis*, said immunogenic composition comprising a purified or recombinant lethal factor (LF) protein or immunogenic fragment thereof and a pharmaceutically acceptable carrier or diluent.

- 18. The immunogenic composition of claim 17 wherein said immunogenic composition comprises a mutated LF protein or an immunogenic fragment of an LF protein and a purified or recombinant B. anthracis PA protein or immmunogenic fragment thereof.
- 19. The immmunogenic composition of claim 18 wherein the mutated LF protein comprises a sequence which is at least 90% identical to a sequence extending from amino acid 1 through amino acid 735 of the sequence set forth in SEQ ID NO:2.
- 25 20. The immunogenic composition of claim 18 wherein the LF protein fragment comprises a sequence which is at least 90% identical to a sequence extending from amino acid 9 through amino acid 252 of the sequence set froth in SEQ ID NO. 2.
 - 22. The immunogenic composition of claim 18 wherein the PA protein comprises a sequence which is at least 90% identical to a sequence extending from amino acid 1 through amino acid 735 of the sequence set forth in SEQ ID NO. 4.

23



- A nucleic-acid based immunogenic composition for preparing a vaccine which protects a subject against lethal infection *B. anthracis*, said immunogenic composition comprising a polynucleotide which encodes a mutated lethal factor (LF) protein or immunogenic fragment of LF protein and a pharmaceutically acceptable carrier or diluent.
- 24. The immunogenic composition of claim 23 further comprising an isolated polynucleotide which encodes *B. anthracis* protective antigen(PA) protein or an immunogenic fragment thereof to the subject, said polynucleotide being operably linked to a promoter which drives expression of the PA protein,
- 25. The immunogenic composition of claim 24 wherein the PA polynucleotide comprises a sequence comprising consecutively nucleotide 610 through nucleotide 2295 of the sequence set forth in SEQ ID NO. 3.
- 26. The immunogenic composition of claim 24 wherein the LF polynucleotide and the PA polynucleotide are on separate DNA constructs.
- 27. The immunogenic composition of claim 24 wherein the LF polynucleotide and the PA polynucleotide are on the same DNA construct.
- A method for inducing an immune response which inactivates the *B. antracis* toxin in an animal, said method comprising administering to the animal an immunogenic composition which comprises an isolated nucleic acid which encodes a mutated *B. anthracis* lethal factor (LF) protein or an immunogenic fragment of LF protein to the subject, said nucleic acid being operably linked to a promoter which drives expression of the mutated LF protein or the immunogenic LF protein fragment.



30

- 29. The method of claim 26 further comprising administering an immunogenic composition which comprises an isolated nucleic acid which encodes *B. anthracis* protective antigen(PA) protein or an immunogenic fragment thereof to the subject, said nucleic acid being operably linked to a promoter which drives expression of the PA protein or immunogenic fragment thereof in the subject.
- 30. The method of claim 28 wherein the method protects the subject from challenge with a dose which is at least 3 times the $\overline{\text{LD}}_{50}$ of the lethal toxin.